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09/380336

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Patentanmeldung Nr. Patent application No. Demande de brevet n°

97200615.9

PRIORITY DOCUMENT

Der Präsident des Europäischen Patentamts:
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Page 2 de l'attestation

Anmeldung Nr.:
Application no.: 97200615.9
Demande n°:

Anmeldetag:
Date of filing: 03/03/97
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
Akzo Nobel N.V.
6824 BM Arnhem
NETHERLANDS

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Diagnostic neodymium (III), ytterbium (III), or erbium (III) ion-ligand complexes

In Anspruch genommene Prioriät(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:
G01N33/58, G01N33/533, C07D311/82

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/BE/CH/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du depôt:

Bemerkungen:
Remarks:
Remarques:

DIAGNOSTIC NEODYMIUM(III), YTTERBIUM(III), OR ERBIUM(III) ION-LIGAND COMPLEXES

The invention pertains to lanthanide ion-ligand complexes, in particular to neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), or erbium(III) ion (Er^{3+}) ligand complexes, to the use of said lanthanide ion-ligand complexes for the manufacture of a diagnostic kit, to a diagnostic kit comprising the same, and to a method of detecting an analyte in a matrix of biomedical interest.

The use of metal chelates as luminescent probes is well-known in the art, and the use of such probes for diagnostic purposes has recently been commercialized, while the state of the art of the technique has been reviewed by P.G. Sammes and G. Yahioglu in Natural Product Reports, Vol. 13, pp. 1-28 (1996). Europium and terbium ions (Eu(III) and Tb(III), respectively), both members of the rare-earth lanthanide metals, are very suitable as luminescent probes because of their long-lived luminescence, which allows for interference-free detection. By using a time delay between excitation pulse and detection optimized for the rare-earth ion, short-lived scatter and background luminescence from the matrix can be effectively removed. Since the absorption coefficients of rare-earth ions are extremely low (1-10 $l.mol^{-1}.cm^{-1}$) direct excitation is very inefficient. In general, excitation therefore requires the aid of a chromophore as sensitizer. For Tb(III) and Eu(III) ions, which emit in the visible part of the spectrum, sensitizers requiring UV excitation have to be applied as a result of energy constraints (the triplet state of the sensitizer should be at least 1000 cm^{-1} above the accepting state of the lanthanide ion). The other lanthanides in chelated forms in solution which exhibit luminescence in the visible part of the spectrum are gadolinium, samarium, and dysprosium ions. The other members of the lanthanide metals are considered to be unsuitable as luminescent probes because they have much smaller gaps between the

excited states and the ground state. When used as a luminescent probe, the lanthanides are coordinated to form complexes with ligands, which complexes often are unstable in aqueous solutions.

Attempts have been made to obtain stable chelates of Eu(III) and Tb(III) ions, for instance, as disclosed in WO 96/00901 wherein complexes of Eu(III) and Tb(III) ions with polynuclear heterocyclic aromatic compounds are described. These chelate-lanthanide complexes may be applied as labels or probes for diagnostics, and are capable of intense luminescence in the visible region. A drawback to these Eu(III) and Tb(III) ion-chelates, however, is the need to sensitize them at a relatively short wavelength (UV or near-UV), which requires expensive lasers. Moreover, the excitation energy used regularly interferes with the biological material which is used for diagnostic purposes, which more often than not is sensitive to light of the UV region. Further it is of advantage to have a metal ion which exhibits a long lifetime in the excited state, which requires minimization of quenching and back-transfer.

It has now been found that when the lanthanide ion-ligand complex comprises neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), or erbium(III) ion (Er^{3+}) as the lanthanide ion, and the ligand comprises or is in contact with a sensitizing moiety which absorbs in the 400-1000 nm region, and preferably in the 400-800 nm region, the complex displays long-lived near-IR luminescence without having the disadvantages of the prior art methods. More preferably, the lanthanide ion-ligand complex further comprises an (immuno)reactant.

When the ligand is in contact with the sensitizing moiety, it may be covalently or ionogenically bonded, or the ligand may be in such close vicinity to the sensitizing moiety that energy transfer between the lanthanide ion-ligand complex and the sensitizing moiety is possible.

These lanthanide ion-complexes can make use of inexpensive 400-800 nm lasers or other light sources, emit luminescence in the near-IR spectrum, have long luminescence lifetimes, high sensitivity, and good stability with respect to the irradiated light and towards the solvents used, especially towards aqueous solutions.

More specifically, the lanthanide ion-complexes according to this invention have long luminescence lifetimes leading to "zero-interference" detection (necessary for diagnostic purposes). Further, the lanthanide ion-complexes according to this invention very efficiently quench the excited triplet state of the sensitizer, which is a major source of singlet oxygen leading to photo-oxidative damage, and therefore enhance stability.
In this respect luminescence is light which is emitted by a compound upon excitation by any means, among them irradiation of laser light.
Luminescence lifetime is the time in which the luminescence emission intensity has decayed to 1/e of its original value.
Luminescence quenching is the process which leads to radiationless deactivation of the luminescent excited state, for instance, as a result of collisions of the excited molecules with species which accept energy from the excited state and dispose of it non-radiatively.

The ligand comprises a complexing moiety which shields the ion from quenching by solvent molecules, in particular water which is generally present in bioassays and contains the OH moiety with particular quenching ability, and provides strong binding of the ion and an anchor for attachment to the (immuno)reactant. The attachment of the (immuno)reactant to the lanthanide ion-ligand complex can be performed by the conventional methods well-known in the art. The ligand further comprises a sensitizing

moiety, which is efficiently excited and able to transfer energy to the lanthanide.

The sensitizing moiety should be as close to the ion as possible to make the energy transfer process more efficient. Alternatively, the ligand may be a complex forming moiety which is in contact with, but not bonded to the sensitizer.

Preferably, the sensitizer comprises a site which can act as the complexing moiety. The sensitizing moiety may be any sensitizing moiety that absorbs light in the visible or near-IR range of the spectrum, i.e. between about 400 and 1000 nm, and more preferably between about 400 and 800 nm. Preferably, the sensitizing moiety is selected from fluorescein derivatives such as fluorexon, eosin, erythrosin, fluorescein, rose bengal, calcium green, and oregon green; triphenylmethane derivatives such as methylthymol blue, xylenol orange, brilliant blue, methyl green, and malachite green; porphyrin derivatives; rhodamine derivatives such as rhodamine 6G, tetrabromorhodamine, and lissamine; phenothiazine derivatives such as thionin and methylene blue; phenoxazine derivatives such as nile blue; coumarin derivatives; acridin derivatives such as acridin orange; (thio)indigo derivatives; carbocyanine derivatives; squaraine derivatives; and (na)phthalocyanine derivatives.

These compounds and derivatives are well-known to those skilled in the art..Coumarin derivatives, for instance, include 2- and 4-coumarins such as coumarin 120, 124, 445, 450, 490, 500, 503, and trifluoromethylcoumarin. Other sensitizers which absorb in the visible region can also be employed.

The ligand may be a composition comprising any compound comprising oxygen, nitrogen, or sulfur moieties which have complexing ability towards Nd(III), Yb(III), or Er(III) ions, in particular polyaminocarboxylic acid, pyridinedicarboxylic acid (dipicolinic acid, DPA), or a derivative thereof, and

a sensitizer selected from fluorescein derivatives such as fluorexon, eosin, erythrosin, fluorescein, rose bengal, calcium green, and oregon green; triphenylmethane derivatives such as methylthymol blue, xylenol orange, brilliant blue, methyl green, and malachite green; porphyrin derivatives; rhodamine derivatives such as rhodamine 6G, tetrabromorhodamine, and lissamine; phenothiazine derivatives such as thionin and methylene blue; phenoxazine derivatives such as nile blue; coumarin derivatives; acridin derivatives such as acridin orange; (thio)indigo derivatives; carbocyanine derivatives; squaraine derivatives; and (na)phthalocyanine derivatives.

Suitable polyaminocarboxylic acids are, for instance, ethylenediamine tetraacetic acid (EDTA), diethylenetriamine pentaacetic acid (DPTA), and triethylenetetraamine hexaacetic acid (TTHA), all basically comprising the aminocarboxylate groups as complexing moieties.

The complexes which are suitable for application as near-IR luminescent long-lived diagnostic labels will, preferably, comprise a functional group which allows the attachment of the compound to an (immuno)reactant, e.g., an isothiocyanate, activated ester (such as N-hydroxysuccinimidyl ester), epoxide, or maleimide group for coupling to amino groups, an amino group for coupling to epoxides, a maleimide or halogenoacetamide group for coupling to thiol groups, a hydrazide group for coupling to aldehydes, and so on.

It has further been found that only neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), and erbium(III) ion (Er^{3+}) exhibit luminescence in the near-IR range of the spectrum ($\lambda_{lum,max}$ = 800 - 1600 nm) with relatively long luminescence lifetimes (τ_{lum}). In this respect the erbium(III) ion (Er^{3+}) is less preferred, because although it exhibits relatively long luminescence lifetimes (τ_{lum}), the luminescence is red-shifted to $\lambda_{lum,max}$ = 1530 nm (see

Tables I and II), which is unfavorable because water absorbs at this wavelength, and also in view of the expensive detectors which are required for detection at 1530 nm.

5 Table I

Luminescence of Ln^{3+}-fluorexon complexes in D_2O (as solvent; conc. 10^{-6} M; λ_{exc} = 500nm), unless indicated differently:

Ln^{3+}	$\lambda_{lum,max}$ (nm)	τ_{lum} (μs)
Present invention:		
neodymium	880,1060,1320	0.3
neodymium (H_2O)	880,1060,1320	0.06
ytterbium	980	6.0
ytterbium (H_2O)	980	1.0
erbium	1530	2.5
erbium (H_2O)	nd[a]	nd
Reference examples:		
praseodymium	nd	nd
samarium	nd	nd
europium	nd	nd
gadolinium	nd	nd
terbium	nd	nd
dysprosium	nd	nd
holmium	nd	nd
thulium	nd	nd

[a] nd = not detectable

Table II

Luminescence of Ln^{3+}-DTPA-fluorescein complexes and Ln^{3+}-DTPA-eosin complexes in H_2O and D_2O (conc. 10^{-6} M; λ_{exc} = 500nm):

Ln^{3+}	τ_{lum} (µs) in D_2O	τ_{lum} (µs) in H_2O
DTPA-fluorescein:		
neodymium	0.5	0.16
ytterbium	1.0	0.61
erbium	1.0	n.d.
DTPA-eosin:		
neodymium	0.5	0.15
ytterbium	1.0	0.6
erbium	1.0	n.d.

n.d. = not detectable

The lanthanides other than neodymium, ytterbium, and erbium do not give luminescence above the detection limit for excitation at 500 nm.

The advantages of the present invention are evident. The near-IR luminescent complexes enable the design of low-cost detection devices. For excitation visible light can be used which can be obtained from inexpensive light sources such as solid-state lasers, light-emitting diodes, or flash lamps. For emission silicon based detectors can be used, and expensive quartz optics are not required.

The near-IR luminescent complexes experience less interference from background signals originating from the sample solution and/or the detection optics, since visible excitation results in less scatter and less background luminescence. Moreover, the luminescence lifetime of the near-IR luminescent complexes is about two orders of magnitude shorter

than that of the prior art Eu^{3+} and Tb^{3+} complexes. On the other hand, the luminescence lifetime of the near-IR luminescent ions is long enough to apply time-gated detection of their emission in order to further reduce the interference from background signals. The shorter lifetime of the near-IR luminescence is advantageous since the flux of luminescence photons is higher, and the number of background photons can be further decreased via the application of a narrower detection gate.

The reduced absorption of the visible radiation used for excitation makes it possible to apply the labels in opaque or strongly scattering matrices, for instance, in direct *in situ* measurements in body fluids and tissues.

Finally, the photochemical stability of the labels towards photo-oxidation is improved, since the triplet state of the sensitizer is efficiently depleted as a result of the energy transfer to the rare-earth ion.

The invention further provides a method for detecting an analyte in a test sample, such as a body fluid or tissue. The method is based on the reaction between the analyte and a specific binding partner for the analyte, and can be an immunoassay, an amplification assay, or hybridisation assay, such as NASBA. The reaction product formed between the analyte and its specific binding partner is detected with a lanthanide ion-ligand complex according to the present invention, which is coupled to an (immuno)reactant. This (immuno)reactant is a compound capable of binding, directly or indirectly, the analyte or the specific binding partner for the analyte. The method can be performed according to different test formats, such as competitive or sandwich types of assays, which are well-known in the art. In the sandwich type of assay the specific binding partner can be immobilized on a solid phase. Various types of solid phases have been described, preferably polystyrene based core-shell latex particles or porous carrier materials are used.

According to a further feature of the invention, a kit is provided for the qualitative or quantitative determination of an analyte in a test sample, said kit comprising:

- a specific binding partner for the analyte,
- a lanthanide ion-ligand complex provided with a labeled (immuno)reactant,

enabling the specific binding partner for the analyte to be coupled to a solid phase.

It is also possible to attach both the specific binding partner and the (immuno)reactant labeled with the lanthanide ion-ligand complex to a solid phase such as a porous carrier material, in which process the specific binding partner is immobilized on said carrier and the labeled (immuno)reactant is coupled to said carrier in such a way that it is freely mobile in said carrier upon contact with test liquid or wash fluid. Such a type of test is comparable with so-called dipstick tests, which are well-known in the art.

Measurement of the lanthanide ion-ligand complexes can be performed by excitation in the visible light range with inexpensive light sources such as solid-state lasers, light emitting diodes, or flash lamps, and detection of the emission by, for example, silicon based detectors. Such light sources and detectors can be used in combination with the device described above.

It is therefore another object of the present invention to provide an apparatus comprising said kit in combination with a light source in the 400-1000nm range, for instance a light emitting diode, and a detector which is suitable for detecting luminescence in the 800-1600 nm range, for instance a silicon or germanium based detector.

In accordance with the present invention, a wide variety of analytes can be measured, including antigens, antibodies, (glyco)proteins, peptides, (oligo)-nucleotides, enzymes, haptens, and polysaccharides.

Specific binding partners for the analyte can be selected from antibodies, antigens, lectins, (oligo)nucleotides, receptors, and substrates.
Examples of components which specifically interact with each other are lectins and sugars, enzymes and substrates, biotin and avidin, DNA and cDNA, RNA and cRNA, DNA and RNA, ligands and receptors.
The (immuno)reactant is a reactant which is capable of specifically binding to the analyte or specific binding partner for the analyte. The (immuno)reactant can be selected from one of the analytes or specific binding partners as specified above. Specific examples are an anti-antibody or protein A.

The invention is further illustrated in the examples.

Examples of direct interaction type complexes: sensitizers with complexing ability towards rare-earth ions.

Example 1

Fluorexon was commercially obtained and used without further purification. The lanthanide ions were added from stock solutions of $YbCl_3 . 6H_2O$, $NdCl_3 . 6H_2O$, and ErCl3 . $6H_2O$ in D_2O or H_2O. Fluorexon, a well-known fluorescence indicator for Ca^{2+} ions, was used to sensitize the near-IR (NIR) emission of trivalent ytterbium, neodymium, and erbium ions. Its absorption spectrum was similar to that of fluorescein, with an absorption maximum at 490 nm.

Solutions were prepared consisting of $5x10^{-6}$ M of the fluorexon and an equimolar amount of lanthanide ions (Yb^{3+}, Nd^{3+}, or Er^{3+}) in D_2O at pD 7. The pD was carefully controlled using an ISFET-based pH meter and concentrated solutions of DCl and NaOD.

The NIR luminescence excitation spectra of the respective Nd^{3+} and Er^{3+} complexes are identical to the spectrum of the fluorexon/Yb^{3+} complex, and

all match the corresponding absorption spectra, with an excitation maximum at 490 nm. These results indicate that energy transfer from the fluorexon to the lanthanide ion is the dominant route to the observed rare-earth ion NIR luminescence and that this process is several orders of magnitude more efficient than direct excitation of the lanthanide. In the used concentration range no luminescence was observed when an absorption band of the rare-earth ion was excited.

Example 2

Methylthymol blue was commercially obtained and used without further purification. The lanthanide ions were added from stock solutions of $YbCl_3 . 6H_2O$, $NdCl_3 . 6H_2O$ and $ErCl_3 . 6H_2O$ in D_2O and H_2O. Methylthymol blue (MTB) was demonstrated to be a luminescence sensitizing agent for ytterbium(III), which emits light in a band around 1000 nm.

Solutions of Yb^{3+} 1x 10^{-5} M and 1x 10^{-5} M MTB in D_2O (pD 5) and H_2O were prepared. When ytterbium ions were added to the solution of MTB, the color changed from yellow to blue, which indicates the formation of a complex between MTB and the ion. The spectra in H_2O are similar, but the luminescence is less intense due to more efficient quenching of the Yb^{3+} excited state by H_2O.

MTB does not sensitize Nd(III). This was demonstrated in an experiment where both fluorexon (see Example 1) and MTB were brought into contact with Nd^{3+} ions. From the absorption spectrum it was clear that both complexed MTB and fluorexon were present, but in the luminescence excitation spectrum (emission at 1060 nm, the principal Nd^{3+} emission line) only fluorexon sensitization was observed.

Examples of indirect interaction type complexes: sensitizers attached to compounds with complexing ability towards rare-earth ions.

Polyaminocarboxylic acids (such as ethylenediamine tetraacetic acid, EDTA, diethylenetriamine pentaacetic acid, DTPA, and triethylenetetraamine hexaacetic acid, TTHA) form stable complexes with lanthanide ions. As examples, sensitizer-modified derivatives of DTPA have been obtained by reaction of its commercially available dianhydride (DTPAA) with an amine. Dye derivatives which absorb in the visible part of the spectrum, like fluorescein and eosin, have been used as amine. The products thus obtained form stable complexes with lanthanides (Yb^{3+}, Nd^{3+}, Er^{3+}), which give intense NIR luminescence upon irradiation with visible light, even in aqueous solution.

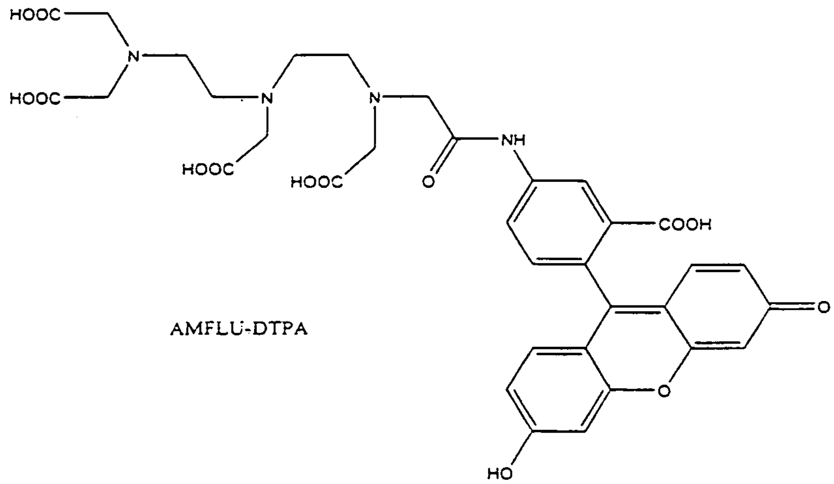
Example 3

DTPAA and 5-aminofluorescein were commercially obtained and used without further purification. The lanthanide ions were added from stock solutions of $YbCl_3$·. 6 H_2O, $NdCl_3$·. 6 H_2O and $ErCl_3$·. 6 H_2O in D_2O and H_2O.

A solution of 15 mg (1.5x 0.56 mmol) of water in 0.5 ml of DMSO was added dropwise to a stirred suspension of 200 mg (1x 0.56 mmol) of DTPAA in 2 ml of DMSO. After 1h, 5-aminofluorescein (100 mg, 0.5x 0.56 mmol) was added. The reaction mixture was allowed to stand overnight at ambient temperature.

The mother liquor was poured into 30 ml of a triethylammoniumacetate (TEAAc) buffer (0.05 M, pH 6.5), and from this solution the AMFLU-DTPA was isolated by means of preparative HPLC over a C_{18}-column. A buffer (TEAAc, 0.05 M, pH6.5)/acetonitrile (MeCN) gradient was applied: 0-5 min 10% MeCN, 5-30 min 10-70% MeCN, 30-32 min 70-100% MeCN, 32-37 min 100% MeCN, 37-40 min 100-0%. The flow rate was 8 ml/min. For

detection use was made of either a diode array-detector or absorbance at a fixed wavelength near the absorption maximum of fluorescein.



AMFLU-DTPA

The fraction at approx. 20 minutes having the typical fluorescein absorption spectrum was collected and concentrated by vacuum evaporation of the eluent. The product precipitated after adding 0.3 M HCl. The yellow powder, AMFLU-DTPA, was collected by centrifugation and washed with dilute acid and acetonitrile.

1H NMR (400 MHz, D_2O/NaOD, 298 K): δ (ppm) 7.97 (1H, d, $^3J_{HH}$ = 2.1 Hz), 7.76 (1H, dd, $^2J_{HH}$ = 8.2 Hz, $^3J_{HH}$ = 2.1 Hz), 7.36 (1H, d, $^2J_{HH}$ = 8.2 Hz), 7.27 (2H, d, $^2J_{HH}$ = 8.9 Hz), 6.65 (2H, d, $^2J_{HH}$ = 8.9 Hz), 6.64 (2H, s), 3.51 (2H, s), 3.34 (2H, s), 3.15 (2H, s), 3.14 (4H, s), 2.86 (2H, m), 2.76 (2H, m), 2.64 (4H, s). For comparison, also the ^1H NMR spectrum of DTPA was measured under the same conditions: δ (ppm) 3.20 (10H, s), 2.72 (8H, s).

All spectroscopic measurements were carried out in buffered D_2O (0.01M Tris/DCl, pD 8). Complex concentrations were in the range 2 - 5 $\times 10^{-6}$ M.

A titration experiment in which the 1060 nm photoluminescence intensity was measured as a function of the Nd^{3+}-to-ligand ratio proved the 1:1 complexation of lanthanide ions with this ligand. The NIR luminescence excitation spectrum (emission at 1060 nm) of the 1:1 AMFLU-DTPA/Nd^{3+}

complex perfectly matches the absorption spectrum of the ligand, indicating efficient sensitization of the lanthanide emission by the fluorescein moiety.

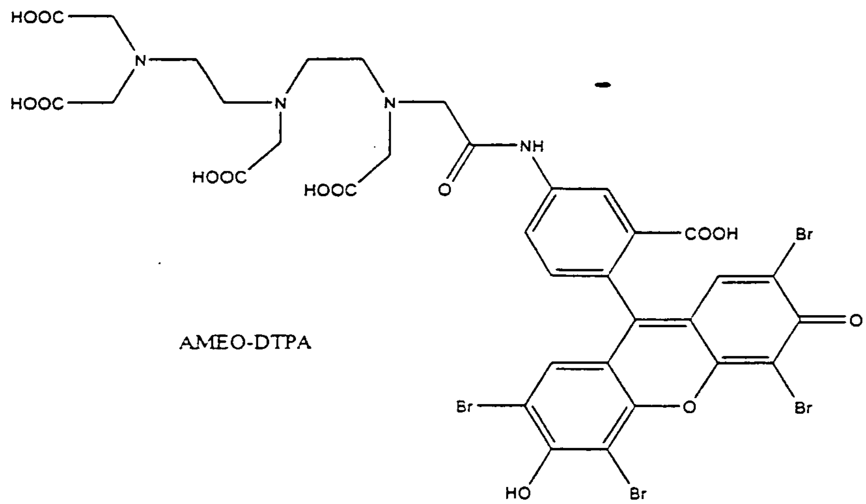
Also the Yb^{3+} and Er^{3+} complexes were prepared by simply mixing stock solutions of the lanthanide ion and AMFLU-DTPA. The NIR luminescence spectra were recorded with an excitation wavelength of 490 nm, the absorption maximum of fluorescein. They all show the sensitized lanthanide luminescence.

Spectra obtained for the complexes clearly show the sensitizing properties of fluorescein: the emission of trivalent ytterbium, neodymium, and erbium at their typical emission wavelengths could be observed when excited at 490 nm, the absorption maximum of fluorescein. In addition, the luminescence excitation spectrum of the Nd(III) emission (at 1060 nm) and the ytterbium emission (at 980 nm) have been recorded and are an excellent match of the absorption (and excitation) spectrum of fluorescein.

Example 4

DTPAA was commercially obtained and used without further purification. 5-aminoeosin was commercially purchased. The lanthanide ions were added from stock solutions of $YbCl_3$·. 6 H_2O, $NdCl_3$·. 6 H_2O and $ErCl_3$·. 6 H_2O in D_2O and H_2O.

The procedure for the preparation of AMFLU-DTPA was repeated using 5-aminoeosin instead of 5-aminofluorescein and yielded AMEO-DTPA, which has its absorption maximum at 515 nm.



AMEO-DTPA

1H NMR (400 MHz, D_2O/NaOD, 298 K): δ (ppm) 7.98 (1H, d, $^{3}J_{HH}$ = 2.1 Hz), 7.80 (1H, dd, $^{2}J_{HH}$ = 8.3 Hz, $^{3}J_{HH}$ = 2.1 Hz), 7.79 (2H, s), 7.46 (1H, d, $^{2}J_{HH}$ = 8.3 Hz), 3.53 (2H, s), 3.36 (2H, s), 3.16 (2H, s), 3.16 (4H, s), 2.88 (2H, m), 2.78 (2H, m), 2.66 (4H, s).

The 1:1 complexation was confirmed in an experiment similar to that used in Example 1. The luminescence excitation and emission spectra of AMEO-DTPA/Ln^{3+} complexes were recorded under the same conditions as the corresponding AMFLU-DTPA complexes and show similar photophysical characteristics.

For the Nd^{3+} and Yb^{3+} complexes, luminescence was also observed in H_2O (Tris/HCl, pH 8).

General: characterization methods

Spectroscopic measurements

Steady state luminescence measurements were performed on a PTI Alphascan spectrofluorimeter, using a 75-W quartz-tungsten-halogen lamp followed by a SPEX 1680 double monochromator for excitation and a PTI 0.25-m single monochromator for separation of the emitted light, detected under an angle of 90°. The emitted light was converted into an electric

signal with a Northcoast 817L liquid nitrogen cooled Germanium detector. For detection a lock-in amplifier (SRS530) was applied; the excitation light was modulated at 70 Hz with an optical chopper. For time-resolved luminescence measurements an Edinburgh Analytical Instruments LP900 system was used, which consisted of a pulsed Xe-lamp followed by a 0.25 m monochromator for excitation and another 0.25 m monochromator positioned at an angle of 90° with respect to the first for separation of the emitted light. The photons were converted into electric signals by means of a Northcoast 817P liquid nitrogen cooled germanium detector (lifetimes >250 ns) or, alternatively, via a Hamamatsu R2658 thermoelectrically cooled photomultiplier tube (lifetimes < 250 ns), and fed to a Tektronix fast digital oscilloscope.

Claims:

1. A lanthanide ion-ligand complex, characterized in that the lanthanide ion is neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), or erbium(III) ion (Er^{3+}) and the ligand comprises or is in contact with a sensitizing moiety which absorbs in the 400-1000 nm region, and preferably in the 400-800 nm region.

2. The lanthanide ion-ligand complex of claim 1 wherein the lanthanide ion is neodymium(III) ion (Nd^{3+}) or ytterbium(III) ion (Yb^{3+}).

3. The lanthanide ion-ligand complex of claim 1 or 2 wherein the complex is further attached to an (immuno)reactant.

4. The lanthanide ion-ligand complex of any one of claims 1-3 wherein the sensitizing moiety comprises a site which can act as the complexing moiety.

5. The lanthanide ion-ligand complex of any one of claims 1-4 wherein the sensitizing moiety is selected from fluorescein derivatives; triphenylmethane derivatives; porphyrin derivatives; rhodamine derivatives; phenothiazine derivatives; phenoxazine derivatives; coumarin derivatives; acridin derivatives; (thio)indigo derivatives; carbocyanine derivatives; squaraine derivatives; and (na)phthalocyanine derivatives.

6. The lanthanide ion-ligand complex of any one of claims 1-4 wherein the ligand is a composition comprising a compound comprising oxygen, nitrogen, or sulfur moieties which have complexing ability towards Nd(III), Yb(III), or Er(III) ions, in particular polyaminocarboxylic acid, pyridinedicarboxylic acid, or a derivative thereof, and a sensitizing moiety selected from fluorescein derivatives; triphenylmethane derivatives; porphyrin derivatives; rhodamine derivatives; phenothiazine derivatives; phenoxazine derivatives; coumarin derivatives; acridin derivatives; (thio)indigo derivatives; carbocyanine derivatives; squaraine derivatives; and (na)phthalocyanine derivatives.

7. The lanthanide ion-ligand complex of any one of claims 1-6 for use in a diagnostic method.

8. A use of the lanthanide ion-ligand complex of any one of claims 1-6 for the manufacture of a diagnostic kit.

9. A method for detection of an analyte in a test sample by a specific binding reaction among the analyte, a specific binding partner for the analyte, and an (immuno)reactant provided with a label, characterized in that the label is the lanthanide ion-ligand complex of any one of claims 1-6.

10. A kit for detection of an analyte in a test sample comprising:
- a specific binding partner for the analyte;
- a labeled (immuno)reactant according to claim 3,

wherein, optionally, the specific binding partner and the labeled (immuno)reactant are attached to a porous carrier.

11. An apparatus for detection of an analyte in a test sample comprising:

- the kit of claim 10;
- a light source in the 400-1000 nm range;
- a detector which is suitable for detecting luminescence in the 800-1600 nm range.

Abstract:

The invention relates to a lanthanide ion-ligand complex, characterized in that the lanthanide ion is neodymium(III) ion (Nd^{3+}), ytterbium(III) ion (Yb^{3+}), or erbium(III) ion (Er^{3+}) and the ligand comprises or is in contact with a sensitizing moiety which absorbs in the 400-1000 nm region, and preferably in the 400-800 nm region. Further, a diagnostic kit is disclosed as well as a method of detecting an analyte in a matrix of biomedical interest through an (oligo)nucleotide, an antigen, or an antibody attached to a material, preferably core-shell latex with specific binding sites, characterized in that the antigen or antibody is labeled with the lanthanide ion-ligand complex and brought into contact with the analyte, after which the analyte with the lanthanide-ion complex is immobilized on the material, and, optionally, residual lanthanide-ion complex is removed, after which the sample obtained is irradiated with light in the 400-1000 nm region, and the emitted light from said sample is detected if said analyte is present in the matrix of biomedical interest.